AD_____

Award Number: DAMD17-00-1-0548

TITLE: Magnetic Resonance Spectroscopy Imaging and Functional Magnetic Resonance Imaging of Neurofibromatosis Type I: In Vivo Pathophysiology, Brain-Behavior Relationships, and Reading Disabilities

PRINCIPAL INVESTIGATOR: Laurie E. Cutting, Ph.D.

CONTRACTING ORGANIZATION: Kennedy Krieger Institute
Baltimore, Maryland 21205

REPORT DATE: October 2003

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 2003	Annual (1 Oct 2002 - 30 Sep 2003)

4. TITLE AND SUBTITLE
Magnetic Resonance Spectroscopy Imaging and Functional Magnetic Resonance Imaging of Neurofibromatosis Type I: In Vivo Pathophysiology, Brain-Behavior Relationships, and Reading Disabilities

5. FUNDING NUMBERS
DAMD17-00-1-0548

6. AUTHOR(S)
Laurie E. Cutting, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Kennedy Krieger Institute
Baltimore, Maryland 21205

E-Mail: cutting@kennedykrieger.org

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

The purpose of this research is oriented towards understanding the reading, language, and articulation deficits associated with Neurofibromatosis Type 1 (NF-1) and relating these deficits to the underlying pathophysiology of NF-1 as revealed by Magnetic Resonance Spectroscopy Imaging (MRSI). A second goal is to determine how differences in activation, as measured by functional Magnetic Resonance Imaging (fMRI), are linked to the cognitive/academic impairments associated with NF-1. A third goal is to further understand how T-2 weighted hyperintensities on Magnetic Resonance Imaging (MRI) scans are related to cognitive/academic impairments associated with NF-1. Each aim addresses the research in terms of pathophysiology and how cognitive/academic functioning of children with NF-1 compares to control groups when examined in both genetic (i.e., sibling) as well as general population (both reading disabled and non-reading disabled) contexts. We hypothesize that abnormalities of NAA, Choline,or their ratios, will exists in the thalamusandwill correlate with language, reading, and articulation deficits in NF-1, defined by "lowering" of the cognitive scores of each child with NF-1 relative to his/her unaffected sibling. For the second goal, we hypothesize that children with NF-1 will activate their brains similarly to reading disabled children during fMRI tasks. For the third goal, we hypothesize that reading, language, and articulation deficits will correlate with the number of brain locations with T2-weighted hyperintesities. Thus, neuroimaging permits the pursuit of furthering our understanding of how the NF-1 gene affects the brain in terms of basic neurobiologic factors (ultrastructural, physiological, and localization) as well as their impacts on cognition (reading, language, and articulation) in NF-1.

14. SUBJECT TERMS
Learing Disability Diagnosis, Neurofibromatosis Type 1, functional Magnetic Resonance Imaging, anatomical Magnetic Resonance Imaging, Magnetic Resonance Spectroscopy Imaging, Reading, and Speech/Language Disorder

15. NUMBER OF PAGES
10

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

TABLE OF CONTENTS

INTRODUCTION

The purpose of this research is primarily oriented towards understanding and documenting the reading, language, and articulation deficits associated with Neurofibromatosis Type 1 (NF-1) and relating these deficits to the underlying pathophysiology of NF-1 as revealed by Magnetic Resonance Spectroscopy Imaging(MRSI). A second goal is to determine how differences in activation, as measured by functional Magnetic Resonance Imaging (fMRI), are linked to the cognitive and academic impairments associated with NF-1. A third goal is to further understand how the brain's visible abnormalities, T-2 weighted hyperintensities on Magnetic Resonance Imaging (MRI) scans, are related to the reading, language, and articulation deficits in NF-1. Each of the specific aims of the research addresses components of the research in terms of pathophysiology and how cognitive/academic functioning of children with NF-1 compares to control groups when examined in both genetic (i.e., sibling) as well as general population (both reading disabled and non-reading disabled) contexts. Based upon previous research findings, we hypothesize that abnormalities of NAA, Choline, or their ratios, will exist in the thalamus; further, that thalamic abnormalities will correlate with language, reading, and articulation deficits in NF-1, as defined by the "lowering" of the cognitive score of each child with NF-1 relative to that of his/her unaffected sibling. In terms of the second goal of this research, we hypothesize that children with NF-1 will activate their brains similarly to reading disabled children during fMRI tasks. In terms of the third goal of this research, we hypothesize that reading, language, and articulation deficits will (as reported for IQ) correlate with the number of brain locations in which T2-weighted hyperintensities are seen. Thus, the use of MRI, MRSI, and fMRI methodology in this research permits the pursuit of further understanding the basic neurobiologic factors (ultrastructural, physiological, and localization) as well as their impacts on cognition (reading, language, and articulation) in NF-1, thus furthering our understanding of how the NF-1 gene affects the brain.

BODY

Research Accomplishments Associated With Each Task: Tasks 1 and 2, which were targeted for years one and two of the grant, have continued to be addressed during the third year of the grant. Task 1 dealt with subject recruitment and data collection (targeted for months 1-26), and included the goals of recruiting patients for participation, screening patients for eligibility, and conducting onsite neuropsychological evaluations and MRSI/fMRI procedures. We have seen a total of 67 patients (19 this year; see chart below), all of whom received neuropsychological testing; of these children, four children with NF-1 (sibling pairs) have participated in MRSI and four children with NF-1, five children with RD, and 9 controls have participated in the fMRI tasks. Task 2 dealt with analyzing MRI data and scoring neuropsychological tests (months 3-26). We have analyzed MRSI data, fMRI data that we have collected and scored neuropsychological tests (including inter-rater reliability) that we have administered.

Tasks 3 and 4 (data entry, statistical analysis, and results/manuscript preparation), which were targeted for the end of year 2 and all of year 3, are currently being addressed as well. We continue to enter MRI and neuropsychological data into the database as we collect MRI and

neuropsychological data. In addition, we presented neuropsychological and related MRI findings at the *International Neuropsychological Meeting* in February 2003 in Honolulu, Hawaii (Cutting, Koth, David, & Denckla, 2003; Crocetti et al., 2003). We are also currently preparing a manuscript for publication on our neuropsychological findings. In addition, we have been asked by the journal *Learning Disabilities Research and Practice* to submit an article on learning disabilities in NF-1 for a special issue that will be published in March 2004. During the 4th year of the grant (we have obtained approval for a no cost extension for another year), we will do some MRI and neuropsychological data collection, but largely focus on analyzing data and preparing manuscripts for publication on both the MRI .

Number of Patients Seen: During the third year of the grant, we have seen 19 children altogether, no children with NF-1, 13 without NF-1, and 6 children with a reading disability (RD). (Although we did not see any children with NF-1, we have 5 children with NF-1 scheduled in the next few months.) Two children in the control group were determined ineligible for the study: one due to psychiatric issues, and one because of reading scores below the 40th percentile. Four children with RD were determined ineligible for the study: three because of reading scores that fell between the 25th and 40th percentile (thus meeting neither RD or control criteria), and one had an IQ below 80. For those children who were found to be ineligible for the study, it did not present a problem because all of them participated fully in the testing and the parents received appropriate feedback (i.e., they will not be included in data analyses). Below is a chart of the participants:

	3rd YEAR	Total to date	GOAL for STUDY
NF-1 W/OUT SIBLING	0	8	20
NF-1 W/SIBLING	0	4	10
NF-1 SIBLING (NON AFFECTED)	0	4	10
CONTROLS	13	31	20
READING DISABILITIES	6	20	30
TOTAL NUMBER SEEN:	**19**	**67**	**95**

Preliminary Findings/Progress:

MRI Findings: Data from the MRSI scans that we have collected have been analyzed by Dr. Barker's group. For the fMRI component of the grant, we have collected data on 18 children for the visuospatial and phonological (reading) fMRI tasks. Below are results for the phonological task (rhyme) and the visuospatial task (analogous to the Judgment of Line Orientation; JLO).

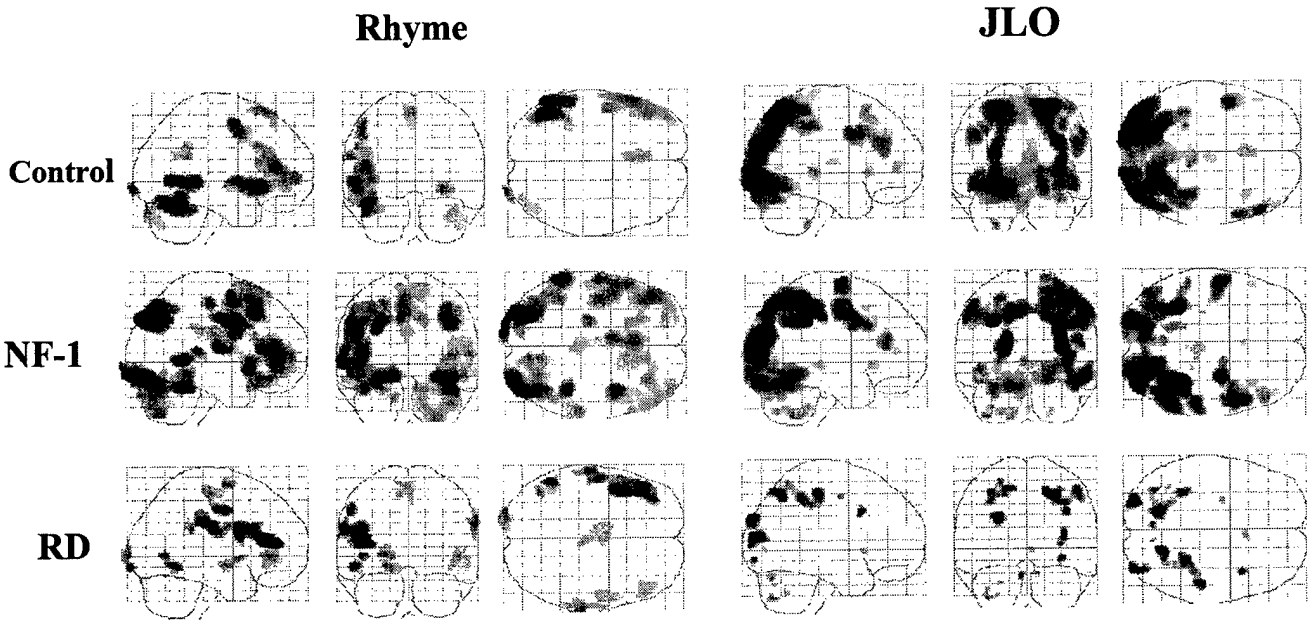

Figure 1. Maps Showing Activation Patterns during Rhyme and Judgment of Line Task (JLO) Tasks.

For the Rhyme task, the control group showed activation in left frontal and temporal lobes, right occipital lobe, and bilateral cerebellum. The NF-1 group showed activation in bilateral frontal, parietal, and occipital lobes, with mimimal activation in the temporal lobes. The RD group showed bilateral frontal activation (L>R), with minimal occipital and temporal lobe activation.

For the JLO task, the control group showed bilateral occipital and parietal lobe activation, with some additional bilateral frontal lobe activation. The NF-1 group also showed bilateral occipital and parietal lobe activation, but showed more bilateral frontal lobe activation than the control subjects. The RD group showed some bilateral parietal and occipital lobe activation, but overall showed lower activation levels compared to the control and NF-1 groups.

Neuropsychological Findings Presented at the International Neuropsychological Society Meeting: Data analyses examining children with NF-1, controls, and children with RD are presented below; we are just beginning to integrate the neuropsychological and MRI findings (this is one of our primary goals for next year during our no cost extension). Analyses on neuropsychological data comparing children with NF-1, children with RD, and Controls (we excluded siblings from analyses) on the language and reading measures show that children with NF-1 have similar deficits to children with RD. Multiple Analyses of Variance (MANOVAs) suggest that children with NF-1 have similar difficulties as children with RD, with both groups showing weaknesses in reading accuracy (decoding), reading comprehension, receptive and expressive language, and some aspects of figurative language. On the other hand, children with NF-1 appear to have some notable differences from children with RD. Unlike children with RD, results suggest that some aspects of inferential language and rate of retrieval (Rapid Naming) are relatively spared in children with NF-1. These findings suggest that intervention for the

associated learning disabilities in children with NF-1 could be able to be tailored to this pattern of strengths and weaknesses (e.g., using their strong rate of retrieval abilities to help remediate language disabilities). Results are listed in Table 1:

Table 1			NF-1	RD	Controls
Ability Test		FSIQ	101.25 ± 17.98 †	100.30 ± 11.13 †	116.07 ± 14.35
		VIQ	103.58 ± 16.74 †	99.30 ± 15.85 †	119.13 ± 15.68
		PIQ	98.75 ± 18.39	102.30 ± 10.73	109.80 ± 15.02
Reading Tests	WIAT	Basic Reading	97.92 ± 11.71 †	83.80 ± 5.49 † ‡	117.20 ± 11.05
		Reading Comprehension	99.58 ± 11.59 †	93.60 ± 11.54 †	115.67 ± 11.95
	WJR	Word Attack	93.92 ± 14.77 †	82.40 ± 7.47 † ‡	115.87 ± 15.28
	GORT-3	Rate	10.75 ± 3.47 †	6.20 ± 2.35 † ‡	14.00 ± 3.98
		Accuracy	8.58 ± 3.06 †	7.10 ± 2.89 †	13.33 ± 3.33
		Comprehension	10.00 ± 3.54 †	6.60 ± 2.84 † ‡	13.27 ± 2.69
Reading-Related Tests	CTOPP	Phonological Awareness	93.00 ± 13.13	92.50 ± 13.87	105.60 ± 12.47
		Phonological Memory	91.50 ± 12.85 †	84.70 ± 8.06 †	102.20 ± 14.72
		Rapid Naming	101.75 ± 11.96	89.50 ± 12.10 $ ‡	106.40 ± 12.92
Language Tests	CELF-3	Receptive Language	95.42 ± 15.83 †	92.50 ± 15.74 †	117.47 ± 19.85
		Expressive Language	97.00 ± 10.39 †	85.30 ± 16.50 †	111.07 ± 17.64
	TLC-E	Ambiguous Sentences	7.92 ± 2.94 †	6.40 ± 2.41 †	10.53 ± 2.90
		Making Inferences	10.00 ± 2.99	8.20 ± 2.82	10.73 ± 1.75
		Figurative Language	8.75 ± 3.33 †	7.30 ± 2.58 †	12.13 ± 2.92

† $p < .05$, NF-1 and RD vs. Controls
‡ $p < .05$, RD vs. NF-1
$ $p < .05$, RD vs. Controls

Problems in Accomplishing Tasks: This year we have had some minor problems accomplishing tasks. In the first year of the grant we had significant "external" impediments that did not allowed us to recruit and evaluate subjects for a total six months (which were: final approval from the US Army Medical Research and Material Command Human Subjects board and shut down of all research at Johns Hopkins Medical Institutions mandated by the Office for Human Research Protections). The second year of the grant went smoothly. However, during the 3rd year of the grant, the HIPPA law was enacted, which required our consent forms to be returned to the US Army Medical Research and Material Command Human Subjects board; this resulted in us being "shut down" for approximately 3 months while these forms were being reviewed by the board. Consequently, we had to cancel approximately 10-15 children who were already scheduled for testing during these months. However, we have had no difficulty this year in terms of interest from the community; the "word has gotten out" about the project and we have numerous families calling to participate. Therefore, barring any further "shut downs" due to human subjects issues, we anticipate a highly productive final year of the grant in terms of recruiting and testing the number of subjects in that we need to fulfill the goals of the grant.

Recommended Changes: So far, we have not encountered any issues that suggest that we should consider changing our goals/procedures of the grant in any manner. We have not encountered any significant obstacles in our research during the third year, and we anticipate a highly productive final year of the grant.

KEY RESEARCH ACCOMPLISHMENTS

- Have identified and established connections with many recruiting sources
- Have seen 67 subjects:
- Have entered all neuropsychological data and conducted statistical analyses.
- Have presented and published abstracts at the *International Neuropsychological Society*.
- Have a manuscript in preparation regarding the neuropsychological findings between NF-1, RD, and Control groups.
- Have been invited to write an article on the learning disabilities present in NF-1 for a special issue of *Learning Disabilities Research and Practice*.
- Have collected and analyzed MRSI data for children with NF-1 (from sibling pairs)
- Collected data for 18 children for the fMRI tasks
- Analyzed data on 18 children for the fMRI tasks

REPORTABLE OUTCOMES

There are several reportable outcomes that have resulted directly from this grant. First, we have presented an abstract on the neuropsychological findings at the *International Neuropsychological Meeting* in February 2003 in Honolulu, Hawaii. Findings showed that both the NF-1 and RD groups showed lower scores that the control group on measures of reading and language, although the NF-1 group performed higher than the RD group on the reading measures (Cutting, Koth, David, & Denckla, 2003). Unlike children with RD, children with NF-1 did not show impairment on rate of retrieval (Rapid Naming), which tends to be predictive of reading fluency. Second, while not directly resulting from the data collected from this grant, this grant has helped support our overall program of research on NF-1 at the Kennedy Krieger Institute. This includes one published abstract, one abstract accepted for presentation, and three publications. One publication, published in *Neurology*, provides detailed analyses of cortical gray and white matter volumes in males with NF-1; lobar (frontal, occipital, parietal, and temporal lobes) and lobar subdivisions (e.g., prefrontal lobe) areas were measured (Cutting, Cooper, Koth, Mostofsky, Kates, Denckla, & Kaufmann, 2002). Findings showed increase in frontal and parietal white matter volumes in patients with NF-1, and frontal gray matter reductions in males with NF-1 who also had ADHD. Another publication examined the growth of "spared" and "impaired" cognitive measures in children with NF-1 as compared to their siblings (Cutting, Huang, Zeger, Koth, Denckla, 2001). Findings indicated that over time children with NF-1 do not "catch up" to their siblings on those measures that were "impaired"; furthermore, there were no significant differences in growth rates between children with NF-1 and their siblings for the "spared" and "impaired" cognitive functions. We also have examined the longitudinal evolution of T2-weighted hyperintensities (UBOs; this manuscript is currently under revision for the *American Journal of Medical Genetics*; Kraut, Gerring, Cooper, Thompson, Denckla, Kaufmann, under revision). Findings showed that the total number of UBO-occupied locations evolved in a non-linear manner, with a decrease between approximately ages 7-12 years, followed by a progressive increase in adolescence. The same pattern was also found for UBO number and/or volume for all regions, with the exception of cerebellar hemispheres. In addition, we have presented a study examining brain volumes of parietal and frontal lobes and neuropsychological functioning in NF-1 and control groups (Crocetti, Cutting, Koth, David, Kates, & Denckla,

2003). Findings showed an inverse relationship between frontal lobe volumes and the Judgment of Line Orientation test, regardless of group membership. Finally, we have an accepted abstract of the results of fMRI of Judgment of Line Orientation in adults that we will be presenting at the *International Neuropsychological Meeting* in February 2004 in Baltimore, MD (Cutting, Clements, Schafer, Mostofsky, Pekar, & Denckla, 2004).

We have applied for funding from the National Institutes of Health (NINDS), to continue our work towards understanding the neurological correlates of the language and reading disabilities reported in children with NF-1. Specifically, we are focusing on refining the knowledge of how to treat children with NF-1 who have reading disabilities as compared to children with idiopathic reading disabilities (IRD). For this research, we propose to determine if children with NF-1 who have reading disabilities respond in the same manner, both neurobiologically (by use of fMRI) and neuropsychologically, to educational interventions known to be highly effective for children with IRD. It is has been established that specialized educational interventions are highly successful for children with IRD, resulting in not only improved reading abilities, but also "normalization" brain activation during reading tasks (using fMRI). We seek to determine if these same interventions will be as effective for children with NF-1.

We have applied for funding from the National Institutes of Health (NIH; R01 HD 044073-01), "Cognitive and Neural Mechanisms of Reading Comprehension". This grant that has been applied for relates to our understanding of idiopathic reading and language disorders, which is relevant to treating the reading and language disorders prevalent in NF-1.

CONCLUSIONS

We have had significant success in reaching the goals of the grant this year. We have seen 19 children and are getting close to our targeted numbers of enrollment. We have also implemented our fMRI paradigms and have collected and analyzed fMRI data on 18 children. Additionally, we have analyzed MRSI data on the 4 sibling pairs that we have seen. We have now addressed many of the goals of the grant. Findings suggest that children with NF-1 show similar difficulties as children with RD, with both groups showing weaknesses in reading accuracy (decoding), reading comprehension, receptive and expressive language, and some aspects of inferential language. On the other hand, children with NF-1 appear to have some notable differences from children with RD. Unlike children with RD, results suggest that some aspects of inferential language and rate of retrieval are relatively spared in children with NF-1. If these findings prove to be true, intervention for the associated learning disabilities in children with NF-1 will be able to be tailored to this pattern of strengths and weaknesses (e.g., strong rate of retrieval abilities may help remediate language disabilities). This grant has also helped support our overall program of research on NF-1 at the Kennedy Krieger Institute. This includes three publications (one published in *Journal of the International Neuropsychological Society*, one in press in *Neurology* and another under revision in the *American Journal of Medical Genetics*), two published abstracts, and one accepted abstract to be presented at the International Neuropsychological Society meeting in February 2004.

REFERENCES

Crocetti, D., Cutting, L.E., Koth, C.W., David, D., Kates W., & Denckla, M.B. (2003). Relationship between parietal and frontal lobe volumes and cognitive functioning in NF-1. *Journal of the International Neuropsychological Society, 9*, 203.

Cutting, LE, Clements, A, Schafer, J., Mostofsky, SH, Pekar, J., & Denckla MB (February, 2004). *Functional MRI of the Judgment of Line Orientation.* To be presented at the International Neuropsychological Society meeting, Baltimore, MD.

Cutting, LE, Cooper, KL, Koth, CW, Mostofsky, SH, Kates, WR, Denckla MB, & Kaufmann, W.E. (2002). Megalencephaly in NF1: Predominantly White Matter Contribution and Mitigation by ADHD. *Neurology,* 59, 1388-94.

Cutting, L.E., Koth, C.W., David, D., & Denckla, M.B. (2003). Comparison of neuropsychological profiles of children with NF-1 and RD. *Journal of the International Neuropsychological Society, 9*, 203.

Cutting LE, Huang G, Zeger S, Koth C, Denckla MB (2001). Specific cognitive functions remain "spared" and "impaired" over time children with Neurofibromatosis Type 1: growth curve analyses of neuropsychological profiles, *Journal of the International Neuropsychological Society, 8,* 838-46.

Kraut, M.A., Gerring, J.P., Cooper, K.L, Thompson, R.E., Denckla, M.B., Kaufmann, W.E. (under revision). Longitudinal Evolution of T2-Weighted Hyperintensities in Children with Neurofibromatosis Type 1. *American Journal of Medical Genetics.*